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ORIGINAL ARTICLE *Reproductive biology*

Overlap of proteomics biomarkers between women with pre-eclampsia and PCOS: a systematic review and biomarker database integration

Gulafshana Hafeez Khan[1,*], Nicolas Galazis[1], Nikolina Docheva[1], Robert Layfield[2], and William Atiomo[1]

[1]Division of Human Development, School of Clinical Sciences, University of Nottingham, Queen's Medical Centre, D Floor, East Block, Nottingham, UK [2]School of Life Sciences, University of Nottingham, Nottingham, UK

*Correspondence address. E-mail: gulafshanahafeez@hotmail.com

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STUDY QUESTION: Do any proteomic biomarkers previously identified for pre-eclampsia (PE) overlap with those identified in women with polycystic ovary syndrome (PCOS).

SUMMARY ANSWER: Five previously identified proteomic biomarkers were found to be common in women with PE and PCOS when compared with controls.

WHAT IS KNOWN ALREADY: Various studies have indicated an association between PCOS and PE; however, the pathophysiological mechanisms supporting this association are not known.

STUDY DESIGN, SIZE, DURATION: A systematic review and update of our PCOS proteomic biomarker database was performed, along with a parallel review of PE biomarkers. The study included papers from 1980 to December 2013.

PARTICIPANTS/MATERIALS, SETTING, METHODS: In all the studies analysed, there were a total of 1423 patients and controls. The number of proteomic biomarkers that were catalogued for PE was 192.

MAIN RESULTS AND THE ROLE OF CHANCE: Five proteomic biomarkers were shown to be differentially expressed in women with PE and PCOS when compared with controls: transferrin, fibrinogen α, β and γ chain variants, kininogen-1, annexin 2 and peroxiredoxin 2. In PE, the biomarkers were identified in serum, plasma and placenta and in PCOS, the biomarkers were identified in serum, follicular fluid, and ovarian and omental biopsies.

LIMITATIONS, REASONS FOR CAUTION: The techniques employed to detect proteomics have limited ability in identifying proteins that are of low abundance, some of which may have a diagnostic potential. The sample sizes and number of biomarkers identified from these studies do not exclude the risk of false positives, a limitation of all biomarker studies. The biomarkers common to PE and PCOS were identified from proteomic analyses of different tissues.

WIDER IMPLICATIONS OF THE FINDINGS: This data amalgamation of the proteomic studies in PE and in PCOS, for the first time, discovered a panel of five biomarkers for PE which are common to women with PCOS, including transferrin, fibrinogen α, β and γ chain variants, kininogen-1, annexin 2 and peroxiredoxin 2. If validated, these biomarkers could provide a useful framework for the knowledge infrastructure in this area. To accomplish this goal, a well co-ordinated multidisciplinary collaboration of clinicians, basic scientists and mathematicians is vital.

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Key words: polycystic ovarian syndrome / pre-eclampsia / biomarker / proteomic / overlap

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age. PCOS can present as infertility, oligomenorrhoea, hirsuitism, acne, hyperandrogenaemia and/or obesity and have metabolic consequences such as an increased risk of hypertension, insulin resistance and type 2 diabetes in later life (Dunaif and Thomas, 2001; Wild, 2002; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Women with PCOS are also known to have an increased risk of obstetric complications including pre-eclampsia (PE), gestational diabetes and preterm birth (Mikola *et al.*, 2001; Boosma *et al.*, 2006; Altieri *et al.*, 2010; Kjerulff *et al.*, 2011; Galazis *et al.*, 2013).

A systematic review performed recently showed that the pregnant women who are known to have PCOS were four times more likely to develop PE when compared with controls (Kjerulff *et al.*, 2011). Although the association between PCOS and PE has been documented, the underlying pathophysiological mechanisms involved are not completely understood; however, it is possible that the raised androgen levels, hyperinsulinaemia and subsequent diabetic and hypertensive susceptibilities in PCOS may act as co-factors (Troisi *et al.*, 2003). Among the various implicating factors, defective placental vasculature appears to be central to the disease (Duckitt and Harrington, 2005).

Currently, there is insufficient evidence to establish causation and to establish screening for patients for these complications, solely based on PCOS diagnosis. There is however a need for research studies into the molecular mechanisms underpinning the link between PCOS and PE. This could facilitate screening in women with PCOS for PE, which could minimize the occurrence of maternal and fetal morbiditiy/mortality associated with PE in pregnant women with PCOS. Proteomic biomarker discovery programmes may address this need.

PE is pregnancy-induced hypertension in association with proteinuria ($>$0.3 g in 24 h) with or without oedema (Royal College of Obstetricians and Gynaecologists, 2006). Virtually, any organ system may be affected. The incidence of severe PE is $\sim$5 in 1000 maternities and is a major cause of poor pregnancy outcomes, including severe obstetric morbidity and maternal and fetal mortality (Royal College of Obstetricians and Gynaecologists, 2006). PE is associated with fetal growth restriction, low birthweight, preterm delivery and respiratory distress syndrome (Royal College of Obstetricians and Gynaecologists, 2006). Pregnant women who are at high risk of developing PE can be identified in the early antenatal period from a comprehensive history enquiring about risk factors, including previous history or family history of PE, age and BMI as well as co-morbidities such as hypertension, renal disease and diabetes (Duckitt and Harrington, 2005). PE is still the second most common cause of maternal mortality as reported by the confidential enquiry into Maternal and Child Health for the triennium of 2006–2008 (CMACE, 2011). The exact pathophysiological mechanism of PE is still unknown.

Proteomics is an emerging discipline which involves the global analysis of protein expression changes (Anderson and Anderson, 1998). There is a common consensus that the information obtained from the protein component of the cell or tissue complements the genomic data. Alterations in protein expression depict biological processes as proteins are the vital elements that control cell function. Proteomic methods are appropriate to detect post-translational alterations. In a literature review of MEDLINE (1966–December 2013), EMBASE (1980–December 2013), ISI web of knowledge (v4.2) and Cochrane (1993–December 2013) databases combining the terms: 'proteomics', 'proteomic', 'pre-eclampsia', and 'PCOS' or 'polycystic ovary syndrome', no studies were isolated, where proteomic biomarkers for PE had been specifically investigated in women with PCOS. However, several studies were identified where proteomic techniques had been used in the study of pregnant women with PE and in women with PCOS as separate entities.

The present study aimed at systematically reviewing the research undertaken using proteomic technologies for the detection of proteomic biomarkers in PE and consider whether any of these biomarkers could be used as candidate biomarkers for identifying the women with PCOS who are at risk of developing PE in pregnancy. This was achieved by performing a comparison of PE biomarkers against previously a published database of all proteomic biomarkers identified so far in women with PCOS (Atiomo *et al.*, 2009). Any biomarkers found to be common to both conditions could be investigated in future studies to understand the mechanisms that link PE with PCOS.

Methods

This study did not involve patient contact; hence, Institutional Review Board (IRB) approval was not mandatory.

Studies eligible for review

MEDLINE (1966–December 2013), EMBASE (1980–December 2013), Cochrane (1993–December 2013) and ISI web of knowledge (v4.2) databases were searched using the terms 'proteomics', 'proteomic', 'pre-eclampsia', 'pre-eclamptic toxemia', 'proteomic biomarker', and 'polycystic ovary syndrome' without any restrictions. Animal studies were not included in the review.

Data abstraction

The original PDFs of studies were acquired through online links to the files obtained from the search results. The references from the studies were manually searched to identify any other relevant studies. The search criterion ended in December 2013. The searches were independently conducted by two of the authors (G.H.K. and N.D.).

Main characteristics of the PE studies

The selected studies were assessed and a record was made of the specific study characteristics including type of study, design, number of participants (*n*), type of proteomic technique used and the exact nature of the sample analysed in each study (whether serum, urine etc.). A list of proteins was created, that were identified to have been expressed differently in women with PE versus controls (normal pregnancy). These parameters are presented in Table I. To improve accuracy, the studies were screened independently by two of the co-authors (G.H.K., N.D.).

Methodological quality assessment

The QUADOMICS tool, which is an adaptation of QUADAS (a quality assessment tool for use in systematic reviews of the diagnostic accuracy studies) takes into account the particular challenges encountered using 'omics' based techniques (Parker *et al.*, 2010) and is recommended in studies using this methodology. The tool was applied to determine the methodological quality of the studies included in this systematic review (Table II). The studies that achieved the score of 12/16 were classified as high quality (HQ), whereas those which scored 11/16 or less were classified as low quality (LQ). The methodological quality assessment was also performed independently by two of the co-authors (G.H.K. and N.D.).

Table I The main characteristics of each study and the proteins differentially expressed in patients with PE compared with normal individuals.

Study	Population		Selection criteria		Proteins identified	Change versus control (↑/↓)	Sample site	Technique used
	n	Mean age ± SD age range	Inclusion	Exclusion				
Epiney *et al.* (2012)	Control, $n = 6$	33.2 ± 2.6	Normotensive pregnant patients	NA	FN1 protein	Decreased	Placenta	LC-ESI-MS/MS
	PE, $n = 4$	38.8 ± 2.3	Systolic blood pressure level ≥ 160 mmHg or a diastolic blood pressure level ≥ 110 mmHg on two occasions and proteinuria ≥3+ on a urine stick or ≥5 g in a 24-h urine specimen (ACOG practice, 2002)		α-Actinin-4	Decreased		
					Actin	Increased		
					Transgelin-2	Decreased		
					Pregnancy-specific β-1-glycoprotein 3	Increased		
					Choriogonadotrophin subunit β	Increased		
					Pregnancy-specific β-1-glycoprotein 2	Increased		
					Protein Si00-A11	Decreased		
					Pregnancy-specific β-1-glycoprotein 4	Increased		
					Phosphatidylethanolamine-binding protein 1	Decreased		
					β-2-microglobulin	Decreased		
					Coagulation factor XIII A chain	Decreased		
					Follistatin-related protein 1	Decreased		
					Malate dehydrogenase	Increased		
					Annexin A2	Decreased		
					Thioredoxin domain-containing protein 4	Decreased		
					Serotransferrin	Decreased		
					C9orf88 variant protein (Fragment)	Increased		
					Cystatin-M	Decreased		
					Polypyrimidine tract-binding protein 1	Decreased		
					40S ribosomal protein S5	Increased		
					Calnexin	Decreased		
					Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A α isoform	Increased		
Buhimschi *et al.* (2008)	38 = PE 21 = control	NA	Severe PE (sPE)	NA	21-aa C-terminus fragment of SERPINA-1	Increased	Urine+ Placenta	SELDI-TOF-MS
	206 (cross-sectional cohort) 19 (longitudnal cohort)		Low risk of PE ($n = 4$) High risk of PE ($n = 15$)		24-aa N-terminus fragment of SERPINA-1	Increased		

Continued

Table I *Continued*

Study	Population		Selection criteria		Proteins identified	Change versus control (↑/↓)	Sample site	Technique used
	n	Mean age ± SD age range	Inclusion	Exclusion				
Park *et al.* (2008)	PE, $n = 18$	32.5 ± 4.7	Blood pressure ≥140/90 after 20 weeks and proteinuria	Multiple pregnancy	Proapolipoprotein A-I	Increased	Amniotic fluid	SELDI-TOF-MS
	Chronic hypertension, $n = 7$	33.0 ± 4.5	Blood pressure ≥140/90 before pregnancy or after 20 weeks	Evidence of intrauterine infection	SBBI42	Increased		Western blot
	Control, $n = 16$	31.6 ± 3.0	No evidence of high blood pressure or proteinuria					
Johnstone *et al.* (2011)	Control, $n = 6$	NA	No evidence of high blood pressure or proteinuria	Smokers Diabetes IUGR Medication other than antihypertensives	α-2-HS-glycoprotein	Increased	Placenta	LC-MS/MS
	PE, $n = 6$		Blood pressure ≥140/90 after 20 weeks and proteinuria		Glutathione S-transferase	Decreased		Western blot
					Peroxiredoxin 6	Decreased		
					Aldose reductase	Decreased		
					Heat shock protein 60	Decreased		
					β-Tubulin	Decreased		
					Heat shock protein 70	Decreased		
					Proteasome, α subunit	Decreased		
					Ezrin	Decreased		
					Protein disulphide isomerize	Decreased		
					Phosphoglycerate mutase 1	Decreased		
					Triosephosphate isomerase	Decreased		
Gharesi *et al.* (2010)	Normal, $n = 5$	24.1 ± 3	No evidence of high blood pressure or proteinuria	Multigravida	Heat shock protein gp96 precursor	Decreased	Placenta	MALDI TOF/TOF
	sPE, $n = 5$	24.3 ± 2.4	Blood pressure, 160 mmHg or higher systolic or 110 mmHg or higher diastolic on two occasions at least 6 h apart, and new onset of proteinuria, 500 mg or more of protein in a 24 h urine collection or 3+ or greater on urine dipstick testing of two random urine samples collected at least 4 h apart	Vaginal birth	Chloride intracellular channel 3	Increased		
					Chain A of enoyl-co-enzyme A hydratase	Decreased		
					Chain A, crystal structure of human Apolipoprotein A-I	Decreased		
					Protein disulphide isomerase	Increased		
					Cu/Zn-superoxide dismutase	Increased		
					Actin, γ 1 pro-peptide	Decreased		
					Peroxiredoxin 3, isoform CRA-a	Decreased		
					HSPA8 (Hsc 70)	Decreased		
					Peroxiredoxin 2 isoform a	Decreased		
					Chain A, Transthyretin	Decreased		
Blumenstein *et al.* (2009a)	Normal, $n = 57$	29.7	Plasma obtained at 20 ± 1 week gestation from the SCOPE biobank. No evidence of high blood pressure or proteinuria	NA	Fibronectin 1 isoform 3 preproprotein	Increased	Serum	LC-MS/MS

	PE (appropriate growth for gestation), $n = 27$	30	Blood pressure $\geq$ 140/90 after 20 weeks on two occasions 4 h apart and combined with either proteinuria or multi-organ complication		Fibrinogen, β chain preproprotein	Increased		Western Blot
	PE (small for gestation age), $n = 12$	30.3	Customized birthweight less than tenth centile for gestational age		Clusterin isoform 1	Increased		
					TTR	Increased		
					Apolipoprotein A-I precursor	Increased		
					Hemopexin	Increased		
					Transferrin	Increased		
Watanabe *et al.* (2004)	Normal pregnant women $n = 80$	30.3 $\pm$ 4.3	No evidence of high blood pressure or proteinuria	NA	Fibrinogen γ chain	Increased	Serum	2-Dimensional electrophoresis MALDI-TOF-MS Western Blot
	PE $n = 80$	30.6 $\pm$ 5.3	Diagnosis of PE was based on the criteria of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy		α-1-antichymotrypsin	Increased		
Blankley *et al.* 2009	Normal pregnant $n = 23$	NA	No evidence of high blood pressure or proteinuria	NA	Clusterin	Increased	Plasma	MALDI TOF/TOF
	PE $n = 23$		PE was diagnosed using standard definitions from the International Society for the Study of Hypertension in Pregnancy		Apolipoprotein B	Increased		
					Inter-α inhibitor H1	Increased		
					Inter-α inhibitor H2	Increased		
					Inter-α inhibitor H3	Increased		
					Complement C6	Increased		
					Complement C7	Increased		
					PAPP-A	Increased		
					Vitamin K-dependent protein Z	Increased		
					Complement C1s	Increased		
					Sex hormone-binding globulin	Increased		
					Clusterin	Increased		
					Coagulation factor X	Increased		
					Coagulation factor V	Increased		
					Insulin-like growth factor binding protein complex acid labile chain precursor (ALS)	Increased		
					Pregnancy-specific B-1 glycoprotein 11	Increased		
					Pregnancy-specific B-1 glycoprotein 1	Increased		
					Vitamin D binding protein[a]	Increased		
					Serum amyloid P-component	Increased		
					Complement C2	Increased		
					Pregnancy-specific glycoprotein 9	Increased		
					Paraoxonase 1	Increased		

Continued

Table I *Continued*

Study	Population		Selection criteria		Proteins identified	Change versus control (↑/↓)	Sample site	Technique used
	n	Mean age ± SD age range	Inclusion	Exclusion				
					Peroxiredoxin-2	Increased		
					Carboxypeptidase N catalytic chain	Decreased		
					Vitamin D binding protein	Decreased		
					α-2-macroglobulin	Decreased		
					Vitronectin precursor	Decreased		
					Afamin precursor (α-albumin)	Decreased		
					Fibronectin 1	Decreased		
					Trypsin-1	Decreased		
					Extracellular matrix protein 1	Decreased		
					Complement C1q	Decreased		
					Plasma protease C1 inhibitor	Decreased		
					Fetuin-A	Decreased		
					Zinc finger protein	Decreased		
					Complement C 4B	Decreased		
Liu *et al.* (2011)	Normal pregnant $n = 5$	28.2 ± 1.8	No evidence of high blood pressure or proteinuria	NA	Serpin peptidase inhibitor, clade A, member 1	Increased	Plasma	LC-MS/MS
	sPE $n = 5$	30.3 ± 2.4	Severe PE was defined as increased blood pressure (≥160 mmHg systolic or ≥110 mmHg diastolic on ≥2 occasions at least 6 h apart) that occurred after 20 weeks of gestation in women with preciously normal blood pressure, accompanied with proteinuria (≥5 g/24 h) or proteinuria of 2+ or more by dipstick measurement		α-2-HS-glycoprotein	Increased		
					AMBP protein	Increased		
					Apolipoprotein E	Increased		
					Apolipoprotein H	Increased		
					Ceruloplasmin	Increased		
					Chorionic somatomammotropin hormone	Increased		
					Clusterin	Increased		
					Coagulation factor X	Increased		
					Complement C1q subcomponent subunit A	Increased		
					Complement C1q subcomponent subunit C	Increased		
					Complement component C8 β chain	Increased		
					Complement component C9	Increased		
					Complement factor H	Increased		
					Complement factor H-related 1	Increased		
					Complement factor properdin	Increased		
					Fibrinogen α chain	Increased		
					Fibrinogen β chain	Increased		
					Fibrinogen γ chain	Increased		

Fibronectin	Increased
Fibulin-1	Increased
Galectin-3-binding protein	Increased
Hyaluronan-binding protein 2	Increased
Kininogen-1	Increased
Lysozyme C	Increased
Serpin peptidase inhibitor, clade F, member 1	Increased
Plasminogen	Increased
Pregnancy-specific β-1-glycoprotein 3	Increased
Pregnancy-specific β-1-glycoprotein 4	Increased
Vitamin D-binding protein	Increased
Vitronectin	Increased
α-2-macroglobulin	Increased
Angiogenin	Increased
Serpin peptidase inhibitor, clade C, member 1	Increased
Apolipoprotein A-II	Decreased
Apolipoprotein B-100	Decreased
Apolipoprotein-L1	Decreased
C4b-binding protein α chain	Decreased
Complement factor H-related protein 1	Decreased
Glutathione peroxidase 3	Decreased
Haemoglobin subunit α	Decreased
Haemoglobin subunit ς	Decreased
Leucine-rich repeat-containing protein 6	Decreased
Mannan-binding lectin serine protease 2	Decreased
Plasma retinol-binding protein 4	Decreased
Platelet factor 4	Decreased
Pregnancy zone protein	Decreased
Serum amyloid A2 protein	Decreased
Serum amyloid A-4 protein	Decreased
Serum amyloid P-component	Decreased
Transthyretin	Decreased

Continued

Table I *Continued*

Study	Population		Selection criteria		Proteins identified	Change versus control (↑/↓)	Sample site	Technique used
	n	Mean age ± SD age range	Inclusion	Exclusion				
	Total = 267		Working Group Criteria on high blood pressure in pregnancy	NA	Matrix metalloprtoteinase-9	Decreased	Decreased	2D-LC-MS/MS
Rasanen *et al.* (2010)	Clinical Cohort:118 Mild PE *n* = 30	28.3			Fibronectin	Increased		
	Severe PE *n* = 30	27.8			Pappalysin-2	Increased		
	Normotensive *n* = 58	28.8			Choriogonadotrophin-β	Increased		
	Preclinical cohort: *n* = 149 Mild PE *n* = 30				Apolipoprotein C III	Increased		
	sPE *n* = 40				Cystatin C	Increased		
	Normotensive *n* = 79				sFlt-1	Increased		
	sPE *n* = 8				Endoglin	Increased		
					Pre clinical cohort			
					Complement factor D	Increased		
					Vascular cell adhesion protein 1	Increased		
					β-2-microglobulin	Increased		
					Cystatin-C	Increased		
					Pappalysin	Decreased		
	Control *n* = 8	NA	Normotensive pregnant women	NA	Heat shock 27 kDa protein 1	Increased	Placenta	LC-MS/MS
Jin *et al.* 2008	PE *n* = 8		Hypertension was defined as a blood pressure >140 mm/Hg (systolic) or >90 mm/Hg (diastolic) on at least two occasions and at least 4–6 h apart after the 20th week of gestation in women known to be normotensive beforehand		78 kDa glucose-regulated protein precursor	Increased		
					Titin	Decreased		
					Prohibitin	Increased		
					Calnexin	Decreased		
					Annexin A1	Increased		
					NADH-ubiquinone oxidoreductase 24 kDa	Increased		
					Chloride intracellular channel protein 3	Increased		
					Smooth muscle and non-muscle myosin alkali light chain isoform 1	Increased		
					Actin α 1 skeletal muscle protein	Increased		
					Keratin 10	Increased		
					Centrosome pr	Increased		

Vascotto *et al.* (2007)	Controls $n = 5$	35 years	Normotensive and women diagnosed with PE	Women with pre-gestational diseases and pregnancy complications	Transthyretin	Increased	Amniotic fluid	MALDI-TOF-MS
	PE $n = 5$	35years						
Myers *et al.* (2013)	*Taining set* Controls $n = 200$	26.8 (6.4)	Normotensive patients	NA	IGFALS	Increased	Plasma	MS-MALDI
	PE $n = 100$	26.6 (6.0)	PE = hypertension associated with proteinuria. Hypertension was defined as a blood pressure >140 mm/Hg (systolic) or >90 mm/Hg (diastolic) on at least two occasions and at least 4–6 h apart after the 20th week of gestation in women known to be normotensive beforehand		MCAM	Decreased		
	Validation set Controls $n = 250$	28.9 (5.3)	Proteinuria = renal excretion of at least 300 mg of proteins in a 24 h urine sample					
	PE $n = 50$	29.7 (5.5)						
Centlow *et al.* (2010)	Control $n = 30$	35.1	No evidence or previous history of PE	Patients with other systemic diseases	Apolipoprotein 1	Increased	Placenta	MALDI –TOF MS/ 2-DPAGE/Western blot
	PE $n = 30$	35.1	PE was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or both, on 2 occasions 4 h apart after 20 weeks' gestation but before the onset of labour, or post-partum, with either proteinuria (24-h urinary protein ≥300 mg or spot urine protein:creatinine ratio ≥30 mg/mmol creatinine or urine dipstick protein ++) or any multisystem complication of PE		Tropomyosin -3	Decreased		
Kolla *et al.* (2012)	Control $n = 6$	30.7 (2.9)	Normotensive	NA	Fibrinogen Fragment D	Increased	Plasma	MALDI-TOF/TOF iTRAQ
	PE $n = 6$	31.7 (1.8)	PE = blood pressure above 140/90 mmHg and proteinuria above 0.3 g/l or rise in blood pressure above 20 mmHg from the first trimester of pregnancy		Clusterin isoform 2	Increased		
					Apolipoprotein A-I	Increased		
					Fibronectin	Increased		
					Angiotensinogen	Increased		
					Galectin 3 binding	Increased		
					Plasminogen	Increased		
					Transferrin	Increased		
					C4 β binding	Increased		
					Haemopexin	Increased		
Blumenstein *et al.* (2009b)	Control $n = 6$		Healthy pregnancy outcome	NA	Vitronectin 75 kDa	Increased		
	PE $n = 6$		PE was defined as systolic blood pressure (BP)		Vitronectin 65 kDa	Decreased	Plasma	

Continued

Table I *Continued*

Study	Population		Selection criteria		Proteins identified	Change versus control (↑/↓)	Sample site	Technique used
	n	Mean age ± SD age range	Inclusion	Exclusion				
			≥140 mm Hg and/or diastolic BP ≥ 90 mm Hg on 2 or more occasions after 20 weeks' gestation but prior to the onset of labour, or post-partum systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mm Hg post-partum on at least 2 occasions 4 h apart, combined with either proteinuria (spot protein to creatinine ratio ≥30 mg/mmol, or 24-h urinary protein ≥0.3 g/24 h, or dipstick proteinuria ≥2+) or any multi-organ complication		a-1-antichymotrypsin (SERPINA3)	Decreased		DIGE Western blot LC-MS/MS
					Kininogen-1	Decreased		

LC-ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometry; 2D-GE/2DE, 2D (gel) electrophoresis; 2D-LC, 2D liquid chromatography; DIGE, differential gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; iTRAQ, isobaric tags for absolute and relative quantification; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MALDI-TOF, matrix-assisted laser desorption time-of-flight; MS, mass spectrometry; *n*, number of participants; PE, pre-eclampsia; Q TOF, quadrupole time of flight; SD, standard deviation; SELDI-TOF, surface-enhanced laser desorption ionization time-of-flight; SRM, selective reaction monitoring; 2D-LC-MS/MS, multidimensional liquid chromatography tandem mass spectrometry; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis. IGFALS, insulin-like growth factor binding protein; MCAM, melanoma cell adhesion molecule.

[a]Vitamin D binding protein is found on the lists of over- and under-represented proteins with different protein database accession numbers. When careful analysis of the peptide data was done manually, it was revealed that the majority of peptides were under-represented in the PE plasma, whereas three peptides matching to a different allele (GC2, T420 K) were at a relatively higher abundance in the PE plasma. This observation also shows the potential of this proteomics workflow to detect differences in isoform expression as well as the potential pitfall of interpreting isoform differences as relative abundance changes if the data are not carefully scrutinized (Blankley *et al.*, 2009).

The PCOS proteomics biomarkers database

The PCOS proteomic biomarkers data has been previously published and validated (Atiomo *et al.*, 2009). A further literature search was however performed on MEDLINE (1966–December 2013), EMBASE (1980–December 2013) and the ISI web of knowledge (v4.2) databases using the following search terms 'polycystic ovary syndrome' and 'proteomic', 'proteomics', or 'proteomics biomarker' without any limits/restrictions. All relevant studies published since the database was last updated in February 2011 were reviewed. One relevant study has since been published, but the updated PCOS database already contained the listed biomarkers found in the paper.

Searching for PE biomarkers in the PCOS biomarker database

A comparison was established between proteomic biomarkers for PE and the updated database of proteomic biomarkers for PCOS. Where overlaps were present, the name of the protein, the original tissue in women with PCOS and PE (where these biomarkers had been identified) and the protein function was recorded.

Results

Proteomic studies of PE

The selection process of the primary studies where proteomic methodologies were used for the identification of biomarkers of PE is shown in Fig. 1. The search generated 58 articles. Review articles, studies that did not use proteomic techniques or studies that did not compare PE with a normotensive (control) group were excluded. Moreover, studies involving animals only, studies presenting protein *m/z* values only rather than protein identifications, or those studies that compared different proteomic approaches were further excluded, leaving 16 primary studies eligible for this review (Watanabe *et al.*, 2004; Vascotto *et al.*, 2007; Buhimschi *et al.*, 2008; Jin *et al.*, 2008; Park *et al.*, 2008; Blankley *et al.*, 2009; Blumenstein *et al.*, 2009a,b; Centlow *et al.*, 2010; Gharesi-Fard

Figure 1 Flowchart showing selection of studies included in the systematic review.

Table II Methodological Quality Assessment using the QUADOMICS Tool.

Quality criteria	Epiney et al. (2012)	Buhimschi et al. (2008)	Park et al. (2008)	Johnstone et al. (2011)	Gharesi-Fard et al. (2010)	Blumenstein et al. (2009a)	Watanabe et al. (2004)	Blankley et al. (2009)	Liu et al. (2011)
1	N	Y	Y	N	N	N	N	N	N
2	N	Y	Y	N	N	Y	Y	Y	N
3	Y	Y	Y	Y	Y	Y	Y	Y	Y
4	Y	Y	Y	N	N	Y	Y	N	N
5	Y	Y	Y	Y	Y	Y	Y	Y	Y
6	Y	Y	Y	Y	Y	Y	N/A	Y	Y
7	Y	Y	Y	Y	Y	Y	Y	Y	Y
8	Y	N	Y	Y	Y	Y	Y	Y	Y
9	Y	N	Y	Y	Y	Y	Y	Y	Y
10	Y	Y	Y	Y	Y	Y	Y	Y	Y
11	Y	Y	Y	Y	Y	Y	Y	N	Y
12	N/A	Y	N	N	N	N	N	N	N
13	Y	Y	Y	Y	Y	Y	Y	Y	Y
14	Y	Y	Y	Y	N	Y	Y	Y	Y
15	Y	Y	N	N/A	N	Y	N	N	Y
16	Y	Y	N	N	N	N	Y	N	N
Total	13	14	13	10	9	13	12	10	11

Quality criteria	Rasanen et al. (2010)	Jin et al. (2008)	Vascotto et al. (2007)	Myers et al. (2013)	Centlow et al. (2010)	Kolla et al. (2012)	Blumenstein et al. (2009b)
1	N	N	Y	Y	Y	N	N
2	Y	N	N	Y	N	Y	Y
3	N	N	Y	Y	Y	Y	Y
4	Y	N	Y	Y	Y	Y	Y
5	Y	Y	Y	Y	Y	Y	Y
6	N/A	Y	N/A	N	Y	Y	Y
7	Y	Y	Y	Y	Y	Y	Y
8	Y	Y	Y	Y	Y	Y	Y
9	Y	Y	Y	Y	Y	Y	Y
10	Y	Y	Y	Y	Y	Y	Y
11	N	Y	Y	Y	Y	N	Y
12	N	N	N	Y	N	N	N

Continued

Table II *Continued*

Quality criteria	Rasanen et al. (2010)	Jin et al. (2008)	Vascotto et al. (2007)	Myers et al. (2013)	Centlow et al. (2010)	Kolla et al. (2012)	Blumenstein et al. (2009b)
I3	Y	Y	Y	Y	Y	Y	N
I4	Y	N	Y	Y	Y	Y	Y
I5	N	N/A	Y	N	Y	Y	Y
I6	N	N	N/A	Y	Y	Y	Y
Total	9	8	12	14	14	13	13

I, description of selection criteria; 2, the spectrum of patients used in each study is representative of the patients who will receive the test in practice; 3, full description of the sample size; 4, adequate description of the procedure and timing of the collection of biological sample with respect to clinical factors; 5, adequate description of handling and pre-analytical procedures—were these the same for the whole sample?; 6, The period between the reference standard and the index test is short enough to reasonably guarantee that the target condition did not change between the two tests; 7, The reference standard is likely to correctly classify the target condition; 8, the whole sample or a random selection of the sample received verification using a reference standard of diagnosis; 9, the patients received the same reference standard regardless of the result of the index test; 10, the execution of the index test is sufficiently described to its permit replication; 11, the execution of the reference standard is sufficiently described to its permit replication; 12, the index test results are interpreted without knowledge of the results of the reference standard; 13, the reference standard results are interpreted without knowledge of the results of the index test; 14, the same clinical data are available when test results are interpreted as it would be when the test is used in practice; 15, any uninterpretable/intermediate test results are reported; 16, the presence of overfitting was most likely avoided; Y, criterion achieved; N, criterion not achieved or not mentioned; HQ, high quality; LQ, low quality; N/A, not applicable.

et al., 2010; Rasanen et al., 2010; Johnstone et al., 2011; Liu et al., 2011; Epiney et al., 2012; Kolla et al., 2012; Myers et al., 2013).

There were a total of 1423 patients and controls in all of the selected studies and 192 different proteomic biomarkers for PE were identified. Six studies investigated placental tissue (Buhimschi et al., 2008; Jin et al., 2008; Park et al., 2008; Centlow et al., 2010; Gharesi-Fard et al., 2010; Johnstone et al., 2011; Epiney et al., 2012), one of which also assessed urine (Buhimschi et al., 2008). Two studies used amniotic fluid (Vascotto et al., 2007; Park et al., 2008), five used plasma (Blankley et al., 2009; Blumenstein et al., 2009b; Liu et al., 2011; Kolla et al., 2012; Myers et al., 2013) and finally, three used serum samples (Watanabe et al., 2004; Blumenstein et al., 2009a; Rasanen et al., 2010). These are summarized in Table I.

Various proteomic techniques that were used in the 16 studies included SELDI-TOF (Surface-Enhanced Laser Desorption Ionization Time-Of-Flight), Mass Spectrometry and MALDI-TOF (Matrix-Assisted Laser Desorption Time-Of-Flight), with Mass Spectrometry and LC-MS/MS (Liquid Chromatography–Tandem Mass Spectrometry) being the most common (Table I).

Assessing the quality of the relevant studies

Out of the 16 studies, 10 were HQ, fulfilling 12 or more of the 16 QUADOMICS criteria (Watanabe et al., 2004; Vascotto et al., 2007; Buhimschi et al., 2008; Park et al., 2008; Blumenstein et al., 2009a,b; Centlow et al., 2010; Epiney et al., 2012; Kolla et al., 2012; Myers et al., 2013). The remaining six studies were LQ, achieving >12 out of the 16 quality criteria (Jin et al., 2008; Blankley et al., 2009; Gharesi-Fard et al., 2010; Rasanen et al., 2010; Johnstone et al., 2011; Liu et al., 2011) (Table II).

Cross-referencing proteomic biomarkers identified in primary studies of PE with database of proteomic biomarkers for PCOS

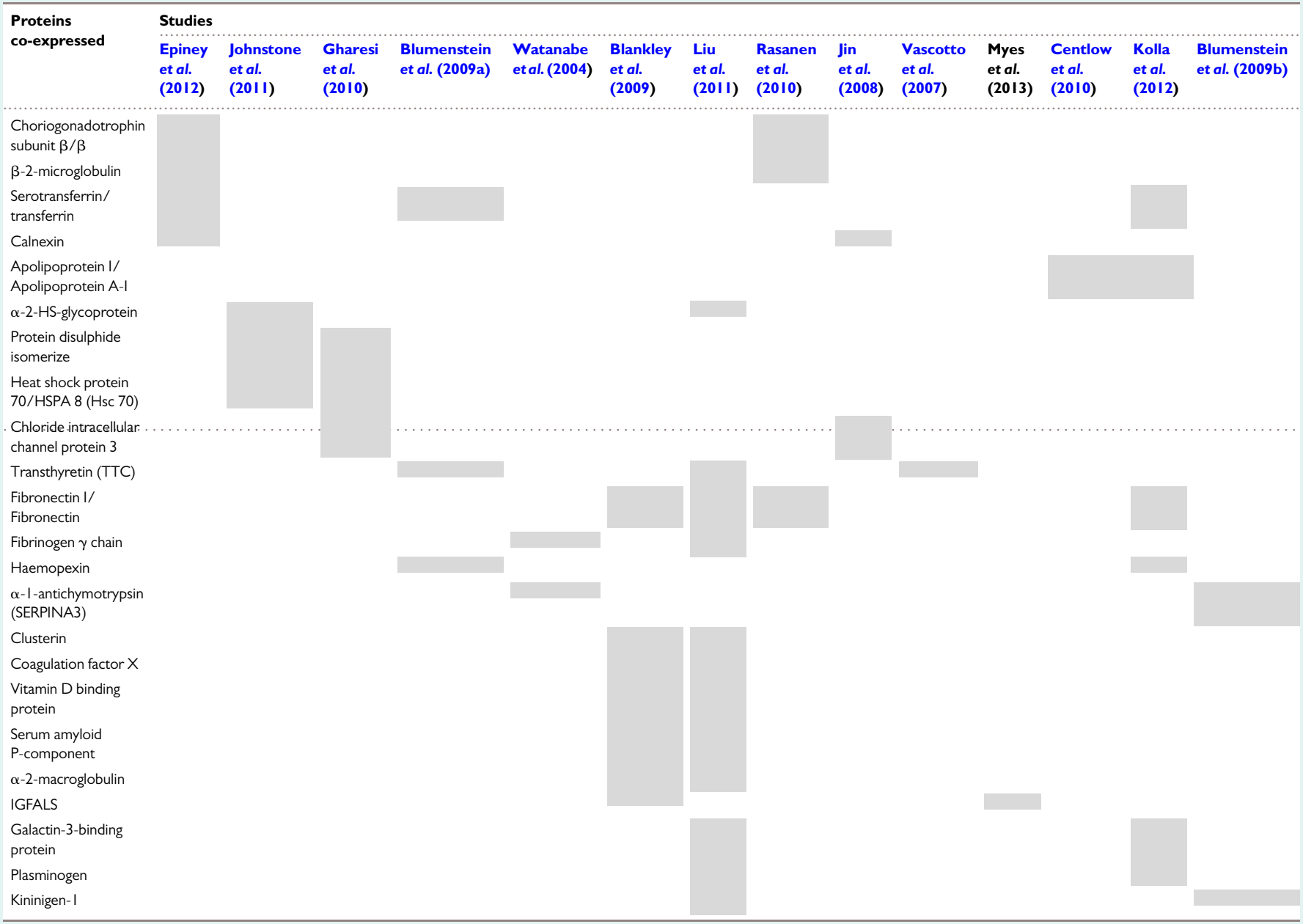
The 192 proteomic biomarkers for PE were cross-referenced with the PCOS database to determine if any were also differentially expressed in PCOS. Five biomarkers were found to be differentially expressed in women with PE and with PCOS compared with controls. Transferrin, fibrinogen α, β and γ chain variants and kininogen-1 were increased and annexin 2 and peroxiredoxin 2 were decreased both in women with PCOS and women with PE. For PE, these biomarkers were found in serum, plasma and placenta, respectively, whereas in PCOS, the biomarkers identified were in serum, follicular fluid, ovarian and omental biopsy, respectively.

Overlaps of the proteomic biomarkers amongst the 16 studies included in this review were also identified and tabulated (Table III).

Discussion

This is the first study that has identified a panel of five proteomic biomarkers which were similarly differentially expressed in women with PE and in women with PCOS. These are transferrin, fibrinogen α, β and γ chain variants, kininogen-1, annexin 2 and peroxiredoxin 2. These findings are of interest but they will need to be validated, and there is a need for future studies that should explore how these proteins interrelate. We have also examined the interactomes of the potential biomarkers using STRING (an online functional protein interaction network; http://string-db.

Table III **Overlaps of the proteomic biomarkers amongst the studies included in this review.**



org/). No evidence for functional interactions between the potential biomarkers (with the exception of the closely related fibrinogen α, β and γ proteins which do interact with each other) was found, although STRING did highlight the co-expression of fibrinogen β and kininogen-1. Thus, at present we are unable to present a pathway that rationalizes how changes in the different candidate biomarkers may relate to one another.

The five proteomic biomarkers identified might clarify the link between PCOS and PE. There is a constant and evolving theme from studies applying proteomic approaches in PCOS about the possible role of immune regulation/inflammation and antioxidants in the pathogenesis of the condition. Similarly, these two pathways have also been implicated in the pathogenesis of PE (Tousoulis *et al.*, 2008; Szarka *et al.*, 2010; Redman, 2011; Yun *et al.*, 2012).

Annexin A2 was down-regulated both in patients with PE and PCOS, although in PCOS, it was found in ovarian biopsies and in PE. it was in placental biopsies. It is known that Annexin A2 is the key physiological receptor for plasminogen on the extracellular surface of endothelial cells (Gugliucci and Ghitescu, 2002). It causes fibrinolysis by accelerating tissue plasminogen activator (Kang *et al.*, 1999) at the endothelial level, via insulin-stimulated plasma membrane translocation of the glucose transporter GLUT-4 (Lennon *et al.*, 2003; Huang *et al.*, 2004). The down-regulation observed in PE tilts the coagulation/fibrinolysis balance towards enhanced coagulation and thrombosis (Gugliucci and Ghitescu, 2002; Ma *et al.*, 2007). We thus postulate that since Annexin A2 is down-regulated both in women with PCOS and PE, it could be a strong candidate for a potential biomarker for the detection of PE in women with PCOS.

Annexin A2 and fibrinogen α, β and γ chains are central in regulating fibrinolysis and thrombosis and their altered expression might represent changes in permeability of the peripheral vessels and vasculature of the various tissues, including ovaries, causing fibrinolysis and abnormal fibrogenesis and thrombosis in PCOS (Gugliucci and Ghitescu, 2002). We speculate that the impaired expression of these proteins may account for the early pregnancy complications such as miscarriage and could impinge upon the cardiovascular system in PCOS patients due to hypofibrinolysis and thrombophilia (Gugliucci and Ghitescu, 2002).

Transferrin was found to be up-regulated in sera of women with PE and PCOS. It is an important β-globulin responsible for transporting iron to various tissues and promoting cell growth and development (Gatter *et al.*, 1983). Transferrin also plays a vital role in pregnancy where it is expressed significantly in the villous syncytiotrophoblasts in women with PE compared with those with normal pregnancies. The cause for this substantial expression in the placentae of pregnancies complicated by either gestational diabetes or PE could be the developing or existing fetal stress (Kralova *et al.*, 2008). Transferrin in high concentrations can inhibit FSH to interact with its receptors on the granulosa cells and this can affect the maturation of oocytes by decreasing the levels of cAMP (Kawano *et al.*, 1995). Transferrin is also known to be a stress/acute phase response molecule. Its upsurge in both women with PCOS and PE could be explained on the basis of the inflammatory constituent of the two conditions.

Kininogen-1 was found to be up-regulated both in women with PE and PCOS in plasma and omental biopsy, respectively. Kininogens play an important role in blood coagulation by helping to position optimally prekallikrein and factor XI next to factor XII and inhibiting the thrombin- and plasmin-induced aggregation of thrombocytes (Wong and Takei, 2013). Moreover, they are a mediator of inflammation and cause increases in vascular permeability, stimulation of nociceptors, and release of other mediators of inflammation (Wong and Takei, 2013). These mechanisms have been implicated in the pathogenesis of both PE and PCOS (Gugliucci and Ghitescu, 2002; Tousoulis *et al.*, 2008; Szarka *et al.*, 2010; Redman, 2011; Yun *et al.*, 2012; Cubedo *et al.*, 2013).

Peroxiredoxin 2 was found to be down-regulated in both PE and PCOS in placental and omental biopsy, respectively (Gatter *et al.*, 1983). In view of the essential role of peroxiredoxin in protecting cells against H_2O_2-induced cell damage and apoptosis, down-regulation in placentae of women with PE emphasizes the role of oxidative stress as an important factor in the development of PE (Cubedo *et al.*, 2013). Furthermore, recent studies have advocated that oxidative stress stimulates androgen-producing steroidogenic enzymes leading to the hyperandrogenism observed in women with PCOS (Burton and Jauniaux, 2004).

As the proteins are the functional units within the cellular environment, analysis of proteomes provide what is presently the finest depiction of disease aetiology at a molecular level.

The discovery of biomarkers poses a challenging task and this is mainly due to the different nature of the samples tested (serum, plasma, urine, tissue). All these samples contain proteins in abundance which reflects their biological activity. It is often thought that tissue biopsy may reflect the disease process more accurately; however, the low invasiveness, low cost and easy sample collection and processing makes the use of body fluids a more attractive option in biomarker studies (Hu *et al.*, 2006). The key to overcome the issues with different samples and analysis is vigilance in sample preparation, state of the art mass spectrometry, careful data processing and cautious data analysis.

One important consideration is that in our analysis, we searched for common biomarkers (to PE and PCOS) but identified from proteomic analyses of different tissues. This raises the question as to whether specific changes in protein (biomarker) expression in, for example, placenta, would be accurately reflected in serum or plasma. Certainly, tissues are characterized by a higher protein complexity than blood, but with the latter is more challenging to interrogate in the initial biomarker discovery phase due to the large dynamic range of blood-derived protein concentrations. Indeed, this is an important question for clinical proteomic analyses in general and not one that has been extensively addressed to date in an evidence-based manner. A few studies relevant to different clinical conditions (such as PE and PCOS) have considered correlations between levels of tissue and circulating biomarkers, with differing results. For example, one study of individuals with abdominal aortic aneurysms found no correlation between levels of amino-terminal pro-peptide of type III pro-collagen between plasma and tissue (Treska and Topolcan, 2000). In contrast, a recent study of non-small lung cell carcinoma demonstrated that GP88 (pro-granulin) is both a tissue and circulating disease biomarker (Edelman *et al.*, 2014), suggesting an association in expression levels. In the context of our own work, it would be of particular interest to perform a future study comparing relative expression levels of proteins in placenta, follicular fluid, ovarian and omental biopsies compared with serum/plasma, and determine whether under conditions where changes in tissue expression occur, such changes are also manifest in the circulation.

The various quantitative and semi-quantitative proteomic techniques used up till now poses a challenge because of the disparate accuracy of the results. We chose to report differential protein expression as either up- or down-regulated which is consistent with previously published systematic reviews of proteomic biomarkers (Baek *et al.*, 2010)

as there is a concern that systematic reviews and meta-analysis are influenced by the clinical heterogeneity. The use of inflammatory markers for diagnosing diseases is another challenge as these markers can also be associated with various other concomitant disease processes. This is a limitation that is known to all biomarker studies of complex diseases (Ling *et al.*, 2011). It is not recommended at this stage that the biomarkers identified in our study are used as conclusive biomarkers of PE and PCOS. Our results provide a framework on which future work can be based and validation studies can be used to better understand the pathophysiological mechanisms linking PCOS and PE.

Proteomic and other 'omic' technologies offer a great prospective for creating new insights into disease aetiology, but it is not without limitations. The relatively slow pace at which research findings have been translated into clinical care is of a concern (Peral *et al.*, 2009). Proteomic techniques have a restricted ability to detect low-abundance proteins, some of which may have diagnostic potential. Moreover, there is a risk of false-positive results as the sample sizes are small (Solomon and Seely, 2006). Emphasis should be placed on data assimilation from primary proteomic studies in order to improve interpretation of research findings and prospective endorsement (Hojlund *et al.*, 2008).

All these issues highlight the fact that there should be more collaboration. This would ensure data synthesis and integration (as in this review) in order to narrow down replicated biomarkers which can be then be validated in subsequent hypothesis-driven research. We see great significance in disseminating our findings to the scientific community as it is vital for the progress in the area of 'omic' research.

Conclusion

Through integrating data from proteomic studies of PE with data from proteomic studies of PCOS, we have for the first time identified a panel of five biomarkers of PE which are common to women with PCOS; these are transferrin, fibrinogen α, β and α chain variants, kininogen-1, annexin 2 and peroxiredoxin 2. If validated, these biomarkers could provide a useful framework on which the knowledge base in this area could be developed. This goal can be achieved by greater collaboration between clinicians, basic scientists and mathematicians.

Authors' roles

G.H.K. and W.A. conceived the idea, did the literature search and supervised the writing of the manuscript. G.H.K. and N.G. did the literature search and wrote the first draft of the manuscript, the flow chart and Table I. G.H.K., N.D. designed Table II and the Venn diagram, and performed the methodological quality assessments. G.H.K, R.L. and W.A. edited various drafts of the manuscript and R.L. advised on data interpretation and analysis and contributed to the revised submission of the manuscript. G.H.K. wrote various drafts of the manuscript, revised the manuscript after review and designed Table I.

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Conflict of interest

None declared.

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